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A STUDY OF THE EFFECTS OF THE SOIL MICROFLORA ON ROOT PATHOGENS IN NATURAL AND AMENDED SOILS

A DISSERTATION

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

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OF MASTER OF SCIENCE

DEPARTMENT OF PLANT SCIENCE

by

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ABSTRACT

Root rots of wheat caused by the three pathogens, Helminthosporium sativum, Fusarium culmorum, and Fusarium graminearum, differed in severity in soils obtained from various geographical regions. The microorganisms in these soils varied in their ability to suppress these pathogens. High soil temperature (30°C.) favoured a microflora, in the Ghanaian soils, that was more antagonistic to H. sativum than that of local soils. This effect was less pronounced at 20°C. A greater number and a large proportion of antagonistic microorganisms present in the microflora of brown and black as compared with grey wooded soils of Alberta was correlated with a reduction of root rot of wheat caused by F. culmorum and H. sativum, respectively. This may indicate that competition for food and space as well as toxic action may limit the activity of plant pathogens in the soil.

Alteration of the soil environment by the addition of amendments derived from plant tissues changed the soil microflora quantitatively and possibly qualitatively, to the detriment of some fungal pathogens. Organic manures, particularly soya bean meal and alfalfa meal, caused a great increase in the numbers

of soil microorganisms. This increase was associated with a reduced incidence of root rot of wheat.

Chemical substances, such as 2,4-D, some soil fungicides and wild oat killing chemicals (avadex and carbyne) were slightly toxic to the soil microflora. This toxicity was indicated by a reduction in numbers of soil microorganisms as compared with that in control soil. The severity of root rot of wheat in soils amended with such chemical substances was not reduced below that of control. Root rot of wheat in soil treated with formaldehyde was less severe than in control soil. In this case the severity of disease was associated with an increased proportion of antagonistic microorganisms in the soil. There was no significant increase in proportions of microorganisms antagonistic to some pathogens in soils treated with the other chemical substances.

INTRODUCTION

The ecology of the soil microflora represents a vast, dynamic interaction of soil microorganisms influenced by a constantly changing environment.

Many plant pathogens are represented in the natural soil microflora (Garrett 1956), while some pathogens may enter the soil temporarily. The survival and multiplication of invading pathogens is dependent on the presence of suitable host plants. The absence of such a specific factor in the environment forces these pathogens to compete with the many saprophytes in the soil. Survival and adaptation to the soil environment may be influenced by the ability of the organism to compete for existing nutrients, to tolerate toxic substances or to produce antibiotics. Biological functions, such as the capacity for rapid reproduction or the ability to antagonize, to lyse or parasitize other microorganisms, may give the microorganism an advantage in securing nutrients.

Food is the basic environmental factor which determines the presence of a soil microflora, while the type of food available influences the nature of the soil microflora. Microorganisms which attack dead plants probably first utilize the water-soluble substances such as pectic materials. These microorganisms

in turn are followed by others which use the less soluble components of the dead plant, leaving the more durable portions. Periodic examination of the soil microflora reveals various types of organisms associated with different stages of organic matter decomposition.

Pathogens that can exist in the soil in the absence of living host plants have an advantage over obligate parasites. In the absence of a suitable substrate, some pathogens may continue to survive by developing a form resistant to the antagonistic products in the soil. Such a resistant stage may be represented by chlamydospores, spores of the perfect and imperfect stages or sclerotia.

Soils from different geographical areas may differ greatly in the type and amount of organic matter and in the physical structure. Such differences are reflected in the quantity and quality of the soil microflora.

Agricultural practices such as rotations go one step further than nature by providing large amounts of certain types of organic matter. Such practices may produce an atypical microflora in the soil. More recently we have seen the increased use of chemicals on agricultural lands. Some of these substances may persist in the soil for a long time and alter the type of microflora during this period.

Other substances remain in the soil for a very short period of time and their influence on the soil microflora may be temporary. Such a temporary change in the microflora may be of sufficient duration to reduce the effects of some pathogens, such as damping-off fungi, which readily parasitize seedling plants during the relatively short susceptible stage.

OBJECTIVES

The investigations reported here have been divided into two parts.

An attempt was made to detect and isolate some of the antagonistic factors present in partially-sterilized, recontaminated and natural soils. Studies were also conducted to determine the effect of selected antagonistic microorganisms on the incidence of disease of some plant pathogens in natural soils from various geographical regions.

The second portion of these studies was designed to test the effects of certain soil amendments on the natural soil microflora. The influence of the soil microflora on the incidence of disease on wheat seedlings caused by some pathogens was investigated. These amendments included some commonly used agricultural chemicals, organic matter of various carbon to nitrogen ratios and glucose.

MATERIALS

CULTURES OF ANTAGONISTIC MICROORGANISMS

Antagonistic microorganisms were obtained by diluting one part of air-dry soil with ten thousand parts of sterile, distilled water. One ml. of the resulting mixture was added to nine ml. of molten Czapek's agar. After incubation for four days at room temperature, the soil dilution plates were sprayed with a water suspension of conidia or minced mycelium of fungal testers by means of a de Vilbiss inhaler. Clear zones appearing after one or two days indicated a lack of growth of the tester around certain colonies of microorganisms, thus denoting antagonists. The selected antagonists were sub-cultured and each retested in association with the pathogen. Successful antagonists were maintained on potato sucrose agar.

Other antagonists used, such as Streptomyces griseus, Trichoderma viride and Bacillus subtilis A-32 were stock cultures maintained at this laboratory.

CULTURES OF PATHOGENIC MICROORGANISMS

Helminthosporium sativum Pamm., King and Bakke (syn. Ophiobolus sativus Ito and Kur.; Cochliobolus sativus (Ito and Kur.) Drechs. ex Dastur. and Bipolaris sorokiniana (Sacc.) Shoemaker)

(Shoemaker 1959), Ophiobolus graminis Sacc. No. 101 (an isolate obtained from a single spore culture), Fusarium culmorum (W.G. Sm.) Sacc. and Fusarium graminearum Schwabe, were obtained from the stock collection of cultures maintained at this laboratory.

Cultures of the pathogens were maintained on potato sucrose agar.

Unless otherwise indicated, the cultures of pathogens and antagonists were applied to the soil as a 5% cornmeal-sterile-soil mixture. This was prepared by mixing 25 gm. of cornmeal with 475 gm. greenhouse stock black soil. Distilled water was added to bring the soil moisture content to about 30%. The mixture was autoclaved for three hours at 15 lb. pressure.

CULTURE MEDIA

Potato sucrose agar: used to maintain stock cultures.

Potato infusion	- 200 gm.
Sucrose	- 20 gm.
Bacto agar	- 15 gm.
Distilled water	- 1000 ml.

Czapek's agar was used in preparing soil dilution plates. Its composition is as follows:

Sucrose	-	30	gm.
Sodium nitrate	-	2.0	gm.
Dipotassium phosphate	-	1.0	gm.
Magnesium sulphate	-	0.5	gm.
Potassium chloride	-	0.5	gm.
Ferrous sulphate	-	0.001	gm.
Agar	-	15	gm.
Distilled water	-	1000	ml.

AMENDMENTS

1. Soya flour: a commercial preparation obtained from a local grocery store.
2. Wheat straw: straw from ripened wheat plants stored indoors over winter was chopped to pass through a 2 mm. sieve.
3. Alfalfa hay: chopped in the same manner as the wheat straw.
4. Starch (soluble): obtained from Merck's laboratories.
5. Peptone: bacto powder.
6. Alphacell: a non-nutritive cellulose prepared by Nutritional Biochemicals Corporation, Cleveland, Ohio.

7. Green manure: green wheat plants (beginning to head) chopped in 1/4 in. lengths. The chopped straw was placed in a cheese cloth bag and washed in running tap water for 1 hour and three rinses of distilled sterile water.

8. Glucose(Powdered): dextro obtained from Fischer.

9. Formaldehyde: obtained from Fischer.

10. 2,4-D: sodium salt 83.6% active. (2,4-Dichlorophenoxyacetic acid)

11. Carbyne: a post-emergence wild oat killer prepared by Spencer Chemical Co., Kansas City.

12. Avadex: a pre-emergence wild oat killer prepared by Monsanto Chemicals, St. Louis, Missouri.

13. D-113: a 50% wettable powder, Control No. 3101 prepared by Chemagro Corp., N.Y. This substance is non-mercuric, soil fungicide.

14. Phenol: crystals.

15. Bayer 22555: 70% active ingredient p-dimethylaminobenzenediazo sodium sulphate. Chemagro Corp., Kansas City. Used as a soil fungicide.

SOILS

Soils were sterilized by autoclaving for 3 hours at 15 lb. steam pressure. All sterilized and partially-sterilized soils, unless otherwise indicated, were obtained from the Plant Science greenhouse stock source of black soil. The other natural summerfallow soils were obtained from sources indicated as follows:

1. Alberta black - University of Alberta Campus, September 1959.
2. Alberta brown - one mile south of Bassano, September 1959.
3. Alberta grey wooded - near park entrance at Cooking Lake, September 1959.
4. Ghana fallow - obtained near Kumasi, February 1958.
5. Ghana forest - obtained from a forest reserve near Kumasi, February 1958.
6. India fallow - obtained by courtesy of Mr. N. Sharan from Agra, Northern India.

The soils from Ghana and India were stored in the refrigerator.

METHODS

Preliminary experiments were conducted to determine the most suitable method of applying the pathogens and antagonists to the soil. The best results were obtained by applying the inoculum (pathogen and antagonist) as a 5% cornmeal-sterile soil mixture. This inoculum was prepared by adding 1 ml. of a water suspension of spores or a square cm. of an agar block (if the fungal culture were non-sporulating) to 500 gm. of steam-sterilized 5% cornmeal-soil mixture. The mixture was incubated 14 days at room temperature and shaken for about one minute daily. The pathogen or antagonist was then applied at the rate of 3.3 gm. of inoculum to 100 gm. of soil. The control treatments received the same rate of a cornmeal-soil mixture but without the microorganism.

Fifteen wheat seeds, surface-sterilized with Perfex (1 part Perfex and 10 parts water) were sown 1 in. deep in 3 in. pots. Each pot contained 150 gm. of soil. All treatments, unless otherwise indicated, were replicated four times. Root-binding was not a serious problem because the plants were harvested after 14 - 21 days.

Optimum moisture conditions of the soil were maintained by setting the pots in tin-foil pie dishes containing about 1/2 in. to 3/4 in. of distilled

water. Distilled water was added to the dishes as needed. All replicates per treatment were placed in one dish.

The washed wheat seedlings were measured for length and rated for disease incidence of the roots and basal portion of the plant. The disease rating system used was as follows:

0 - no disease evident.

1 - trace amount of disease (brown discolouration of roots or of the underground portion of the stem).

2.- more discolouration of parts mentioned in 1.

3 - fairly severe lesioning of roots and base of stem.

4 - plant root and stem severely lesioned and leaves turning yellow.

5 - plant dead as a result of the disease.

It was generally noted that a low incidence of disease was associated with increased height of plant and a more vigorous root system. The incidence of disease due to all pathogens was recorded in the same manner.

Maximum infection by O. graminis was obtained by maintaining the soil temperature at 60°F. from seeding until emergence of the wheat seedlings.

DETECTION OF ANTAGONISTS IN THE SOIL BY MEANS OF SOIL DILUTION PLATES

A method used by Herr (1959) was adopted in these investigations. This method is outlined briefly. Five gm. of soil (air-dry weight) were added to 50 ml. of distilled water and shaken intermittently for 30 minutes. One ml. of this suspension was mixed with 100 ml. of sterile distilled water. This serial dilution was repeated until the desired concentration was obtained. One ml. of this dilution of soil suspension was added to 5 ml. of water agar maintained at 40°C. The mixture was agitated and poured onto 10 cc. of solid water agar in a Petri plate. After incubation for four days, pin point size colonies became visible. Five ml. of molten Czapek's agar containing a large number of spores of a pathogen were added as a third layer. The cultures were incubated at room temperature for two to three days after which time all colonies that possessed antagonistic properties showed up with a clear zone immediately about the colony. This is illustrated in Figure 1.

A dilution factor of 1:10,000 for natural soils was sufficient to give clear counts of the total numbers of microorganisms on a plate. For ease in counting microorganisms on dilution plates, amended soils were diluted to a greater extent -- up



Figure 1. Actinomycetes and bacterial colonies showing antagonism towards H. sativum on soil dilution plates.

to 1:100,000,000. All treatments were replicated six times, unless otherwise indicated.

PREPARATION AND USE OF AMENDED SOILS

Organic amendments were used in low concentrations of 0.5 to 5% of the weight of the soil. These concentrations are indicated for each experiment.

All substances, with the exception of Carbyne, Avadex and formaldehyde, were applied as dust. Carbyne and Avadex were applied as 6 cc. of 1.67% and 4 cc. of 0.42% solutions, respectively, to 600 gm. of soil. Formaldehyde was applied by placing a pot with 600 gm. of soil in a tray containing about 1 in. of formaldehyde solution of the appropriate concentration. After 12 hours, the saturated soil was spread on paper to dry. These treated soils were incubated ten days in order to establish an altered soil microflora. The amended soils were treated with 3.3% (by weight) cornmeal-soil inoculum of pathogens or antagonists and sown to wheat.

All labels, pots and tin foil pie dishes were autoclaved for 20 minutes at 15 lb. pressure.

The survival of four pathogens in three Alberta soils was determined by removing at intervals a certain amount of pathogen-inoculated soil from a bulk source kept in a crock and sowing these soils to

wheat. The periods of time will be indicated in the appropriate section dealing with the experiment. The soil in the crocks was kept loosely covered with a plastic sheet so that watering was necessary only once in eight weeks. The soil was thoroughly mixed each time a sample was removed.

PREPARATION OF RECONTAMINATED SOILS

Since there was insufficient soil from Ghana and India for pot experiments, the microflora from these sources was established in steam-sterilized black soil or in a sterile alphacell base material in a manner similar to that described by Henry (1931). Ten grams of natural soil were added to 100 grams of sterilized soil. The mixture was brought to optimum moisture content (for wheat seedling development) and incubated for seven days at the temperatures indicated under the appropriate experiments. The soil and alphacell mixtures were shaken one minute daily.

The alphacell base material consisted of a 1:5 mixture of alphacell and washed white sand. To this mixture, 30 cc. of sterilized solution were added. The soil solution was prepared by extracting black natural soil with sterile distilled water (1 litre for 1 kg. soil) and sterilizing the extract

by passing it through a sintered-glass filter.

Both the sterile alphacell and soil were contaminated with 4% (by weight, air-dry) non-sterile soil and incubated for seven days at 25°C. The mixtures were shaken one minute daily. The pathogen was added at the rate of 4% by weight of cornmeal-soil inoculum and the mixtures were then sown with wheat.

The virulence of the pathogens against wheat plants in every experiment was checked by growing wheat in the presence of the pathogen. This was done to guard against the use of an attenuated strain of the pathogen.

EXPERIMENTS AND RESULTS

LITERATURE REVIEW

The natural microflora of any soil is in a state of dynamic equilibrium for a given set of environmental conditions. Such a microflora includes soil-borne plant pathogens. The influence of the natural soil microflora which is part of the environment of a pathogen is demonstrated by comparing the effect of a pathogen on its host growing in sterile and in natural soil. Henry (1931) and Ludwig and Henry (1943) demonstrated the adverse effect of the microflora of natural soils on the survival of H. sativum and O. graminis. They also demonstrated the remarkably rapid recolonization of sterile soil following the introduction of a trace amount of natural soil. Henry (1931) showed that a mixture of bacteria, actinomycetes and fungi was only slightly more effective than fungi alone in reducing the effect of H. sativum on wheat in sterile soil.

Morton and Stroube (1955) noted that about 3.5% of the fungi in the soil showed antagonism to Sclerotium rolfsii as compared to 1.7% and 0.2%, respectively, for actinomycetes and bacteria. Luke and Connel (1954) noted that 16% of all fungi isolated

from sugar cane soils showed antagonism to Pythium, whereas only 3.6% of the bacteria and actinomycetes showed antagonism to this plant pathogen.

Other workers have reported that actinomycetes or bacteria may be chiefly responsible for the antagonism in the soil. Stessel, Leben and Keitt (1953) found that 80% of the microorganisms antagonistic in vitro to Glomerella, Colletotrichum, H. sativum and Verticillium were actinomycetes. Schatz and Hansen (1948) stated that actinomycetes as a group contain a greater proportion of antagonists than fungi and bacteria. The importance of these organisms as antagonists was indicated in a study made by Landerkin et al. (1950) of the antibiotic activity of actinomycetes from soils of northern Canada.

The antagonistic microflora encountered in these investigations were chiefly actinomycetes and bacteria. Czapek's medium may have been selective for actinomycetes and bacteria in platings of soil dilutions. The use of in vitro methods would, of course, always be a limiting factor in determining the antagonism of a microorganism to a given pathogen. The growth, and metabolism of soil microflora on agar may be atypical to that in the soil.

A microorganism may show antagonistic properties towards certain pathogens *in vitro*, but may be unable to exert the same effect in the soil (Wood and Tviet 1955). Some microorganisms may be antagonistic to a pathogen in the soil under certain environmental conditions, such as a high pH, and not under others (Wright 1956). Such cases are illustrated by the increased antibiotic production of microorganisms in amended soils (Gottlieb, Siminoff and Martin 1952).

An attempt was made to examine individually the causal agents of such antagonism. The demonstration of the ability of one organism to inhibit another on agar is now classical, but many modifications of this method exist (Jeffreys 1948, Stessel 1951). A fairly simple method, employing the triple layer of agar dilution plates, for detecting antagonistic microorganisms in soil, has been described by Herr (1959). In addition to antagonistic microorganisms isolated, others known to be effective against a wide range of microorganisms were *Trichoderma* spp., *Penicillium* sp., *Gliocladium* sp., *Streptomyces griseus*, *S. antibioticus* and *Bacillus subtilis* (Wood and Tviet 1955).

The production of antibiotics by specific microorganisms in the soil and in artificial media is

well known. Wright (1955) recorded the production of griseofulvin by Penicillium nigricans in sterile soil. Recontamination caused a reduction of antibiosis in sterile soil. Weindling (1934) studied a lethal principle effective in the antagonistic action of Trichoderma on Rhizoctonia solani and other soil fungi. Later, the same author isolated toxic substances from culture filtrates of Trichoderma (Weindling 1936). Some microorganisms produce antibiotics only under specific conditions. Wright (1954, 1956) stated that T. viride can produce gliotoxin only in acid soil. The ability of this fungus, however, to produce a wide range of antibiotics may partly explain its well known antagonistic properties in the soil. Another antibiotic isolated from this fungus by Brian and McGowan (1945) was a highly fungicidal substance called viridin. B. subtilis also has been shown to produce a number of antibiotics, such as bulbiformin, subtilin and toxymycin (Stessel, Leben and Keitt 1953, Vasudeva et al. 1958). The ability of this bacterium to produce a wide range of antibiotics led Vasudeva to investigate the effect of this bacterium on Fusarium udum, the causal agent of pigeon pea wilt (Vasudeva et al. 1952) and on Alternaria solani (Vasudeva 1954).

The production of antibiotics by microorganisms has been shown to occur most readily in sterile soil and other suitable media, but some workers have found that certain antagonistic microorganisms may produce antibiotics in natural soil (Wright 1952, Gottlieb and Siminoff 1952). Antibiotics detected in natural soils are possibly more resistant to microbial or chemical decomposition and may thus be more effective in reducing root diseases of plants. Most antibiotics persist only a short period of time in natural soil. Peterson (1952) found that actidione could persist in natural soil for several weeks, whereas streptomycin was inactivated almost immediately. It is likely that many antibiotics are produced in natural soils but in such small amounts as to remain undetected. This small amount of antibiotic, however, may be sufficient to influence a germinating spore (Stevenson 1956). Jeffreys (1952), and Martin and Gottlieb (1952) showed that some antibiotics were more stable at specific pH values or in different types of soil. Martin and Gottlieb (1952) found that terramycin and aureomycin were inactivated by adsorption onto illite and bentonite clays. Other antibiotics, such as actidione and clavacine, were not removed from aqueous solutions by the soil, nor were they adsorbed onto clay (Gottlieb et al. 1952).

Some microorganisms also may inhibit the growth of plant pathogens with relation to nutrient conditions. Skinner (1956) noted that Streptomyces albidoflavus arrested the growth of F. culmorum on agar and moist sand containing 10 gm. glucose per litre. By reducing the sugar concentration, the fungus continued to grow in the presence of the actinomycete. The fungus and actinomycete grew together on a variety of natural organic matter, but only when dried grass was used did the actinomycete arrest the growth of the fungus by means of antibiotic action.

Many workers have reported the parasitic action of fungi on other fungi. Slagg and Fellows (1947) described the parasitism of O. graminis by T. lignorum. Campbell (1956) found that Myrothecium verrucaria was only slightly inhibitory, but was actually parasitic upon H. sativum.

EXPERIMENTAL RESULTS

(1) The antagonistic effect of some microorganisms on several plant pathogens

The effect of several actinomycetes on the survival of O. graminis 101 was determined in sterile and non-sterile Alberta black soil. The following actinomycetes were used because of their marked antagonism to O. graminis on potato dextrose agar:

Actinomycete No. 284 - isolated from soil in Ghana

Actinomycete No. 453 - isolated from soil in Ghana

Actinomycete No. 380 - isolated from soil in Ghana

S. griseus - stock sulture at this laboratory.

The actinomycetes and pathogen were cultured on 5% cornmeal-sterile soil for seven days and then were mixed at the rate of 5 gm. per 150 gm. soil (3.3% by weight). The inoculated soil was placed in 6 in. pots and 50 surface-sterilized Red Bobs wheat seeds were sown. The number of seedlings emerged, their height at 14 days, their disease rating, and average percentage of healthy plants from four replicates are presented in Table I.

TABLE I. THE INFLUENCE OF SOME ACTINOMYCETES ON THE INFECTION OF WHEAT SEEDLINGS BY OPHIOBOLUS GRAMINIS 101 IN STERILIZED AND NATURAL (NON-STERILE) SOIL.

	NON-STERILE SOIL				STERILE SOIL			
TREATMENT*	PERCENT EMERGED	HEIGHT IN INCHES	DISEASE RATE	PERCENT HEALTHY	PERCENT EMERGED	HEIGHT IN INCHES	DISEASE RATE	PERCENT HEALTHY
1	76	7.9	0.9	63.9	90	10.5	0.4	70.5
2	74	5.7	2.7	9.2	92	7.5	2.9	7.4
3.	79	5.6	2.2	12.4	87	7.6	3.1	2.4
4	74	5.7	3.0	1.3	91	6.8	3.6	2.8
5	67	5.8	2.4	0	91	6.8	4.1	0
No pathogen	74	9.7	0	100	93	10.4	0	100

*Treatment 1. O. graminis and S. griseus

*Treatment 2. O. graminis and Act. 380

*Treatment 3. O. graminis and Act. 284

*Treatment 4. O. graminis and Act. 453

*Treatment 5. O. graminis

Note: Percent emergence is high because O. graminis is more pathogenic to wheat seedlings after emergence than before emergence.

The results presented in Table I show that the effectiveness of the pathogen alone in sterile soil was greater than in a natural soil. These results emphasize the antagonistic effect of S. griseus against O. graminis. Several of the other actinomycetes which were selected because of their antagonistic properties to O. graminis on agar, failed to demonstrate the same degree of antagonism in the soil.

The incidence of disease on wheat seedlings due to H. sativum growing in soil infested with B. subtilis A32, T. viride and an actinomycete No. 284, was studied. In this experiment, the cultures of antagonists and pathogen were applied to the soil as concentrated spore suspensions in sterile distilled water. Spores of the three antagonists were applied at the rate of 10 cc. per 750 gm. of soil and the pathogen was applied at the rate of 5 cc. per 450 gm. of soil. The results of these tests are shown in Table II.

TABLE II. THE INFLUENCE OF SOME ANTAGONISTIC MICROORGANISMS (FUNGAL, BACTERIAL AND ACTINOMYCETE) ON THE INFECTION OF WHEAT BY HELMINTHOSPORIUM SATIVUM IN STERILE AND NATURAL (NON-STERILIZED) SOIL.

	STERILE SOIL				NON-STERILE SOIL			
	H. SATIVUM		NO H. SATIVUM		H. SATIVUM		NO H. SATIVUM	
ANTAGONIST	PERCENT EMERGED	DISEASE RATE	PERCENT EMERGED	DISEASE RATE	PERCENT EMERGED	DISEASE RATE	PERCENT EMERGED	DISEASE RATE
Act. 284	96	0	100	0	38	0.1	77	0
B. subtilis	96	0	87	0	62	0	80	0.1
T. viride	93	0.1	83	0	47	0	93	0
None	49	3.5	90	0	60	0.5	67	0

These results indicate that the antagonists used in this experiment were effective in reducing the activity of H. sativum in sterile soil only. Ungerminated conidia of H. sativum applied to natural soil failed to parasitize the wheat seedlings. This would suggest the presence of toxic products in natural soils.

The presence of antagonists without H. sativum in natural soil increased the germination of wheat, possibly by inactivating the weak parasitic seed-borne fungi or bacteria.

(2) Recolonization of sterile soil

The investigations of Henry (1931) demonstrated the rapid recolonization of sterile soil by trace amounts of natural soil. More recently others have attempted to control the reinfestation of sterile soil by the introduction of non-pathogenic microorganisms. Ferguson (1953) found that species of Chaetomium, Pestalotia, Myrothecium, Penicillium and Trichoderma, when added to sterile soil containing Rhizoctonia solani, greatly reduced the effects of this pathogen.

Johnson (1952) exposed sterile soil to aerial contamination for varying periods of time and noted that antibiosis towards Pythium arrhenomanes increased with time. Ludwig (1939) and Ludwig and

Henry (1943) showed that unprotected sterile soil was rapidly recolonized by T. viride. Its activity was indicated by the reduction of disease incidence on wheat seedlings due to O. graminis.

The physical structure of sterilized soils also may be altered (Newhall 1955). This may have some effect on the type and amount of microflora that may recolonize such soils.

Steam-sterilized soil was recontaminated with various amounts of natural soil. The recolonization of these soils by microorganisms was detected by the reduction of the disease of wheat on seedlings due to H. sativum. Six hundred grams of sterile soil were inoculated with 6×10^{-6} gm. to 6×10^{-2} gm. of natural soil. The inoculated soil was incubated for one and five days, respectively, before 20 gm. of H. sativum inoculum were added to 600 gm. of contaminated soil. The soil was placed in sterile 3 in. pots and sown to wheat. The results of these tests are shown in Table III.

TABLE III. THE EFFECT OF STERILE SOIL, RECONTAMINATED BY VARIOUS AMOUNTS OF NATURAL SOIL, ON THE INCIDENCE OF ROOT ROT OF WHEAT DUE TO H. SATIVUM WHEN THE PATHOGEN WAS ADDED ONE AND FIVE DAYS FOLLOWING RECONTAMINATION.

AMOUNT OF NATURAL SOIL ADDED TO STERILE SOIL	ONE DAY		FIVE DAYS		CONTROL	
	PERCENT EMERGED	DISEASE RATE	PERCENT EMERGED	DISEASE RATE	PERCENT EMERGED	DISEASE RATE
None	83	1.3	84	1.3	87	0
$6x10^{-6}$ gm.*	75	1.4	77	2.1	88	0
$6x10^{-4}$ gm.*	60	1.3	77	0.4	86	0
$6x10^{-2}$ gm.*	68	1.4	83	0.3	80	0
$6x10^{-2}$ gm.**	77	2.1	72	0.3	95	0

* Dilution method

** Dry weight

The results in Table III suggest that sterile soil which was recontaminated required more than 24 hours to re-establish a soil microflora that would reduce the severity of disease on wheat seedlings due to H. sativum. The amount of natural soil required for this recolonization was about 0.0006 gm. per 600 gm. of sterile soil.

(3) Recolonization of partially-sterilized soil

Singh and Crump (1953) partially sterilized soil with formalin and steam. They found that the numbers of bacteria increased and the numbers of amoeba decreased in formalin-treated soil, while the numbers of bacteria and amoeba increased in steamed soil. These effects were recorded during a period of six months before the soil populations approached those of the control. Mollison (1953) studied the partially-sterilized soils of Singh and Crump and found that the fungal populations had been much reduced. This effect lasted for at least 25 months. He also found that the predominant fungus in formalin-treated soil was a Trichoderma sp., whereas a greater variety of fungi was found associated with steamed soil. Newhall (1955) quoted the works of Kurzweil in 1943 who stated that actinomycetes can tolerate steaming for some hours. Warcup (1951) compared steam- and formalde-

hyde-treated, 25-year old forest nursery soil of high pH and found that more fungal species recolonized the steamed than formaldehyde-treated soil. The dominant fungus in the formaldehyde-treated soil was T. viride, while in the steamed soil, Mortierella, Phoma and Coniothyrium were most common.

Newhall (1955) stated that heating the soil resulted in the production of adverse growth effects which were the products of time and temperature. He stated that toxicity was counteracted by an abundance of phosphate applied before or after steaming, or by the addition of untreated soil to the steamed soil. He also found that fewer toxic products were formed in fumigated than in heated soil. Nitrification may be inhibited and ammonification may be accelerated in fumigated soils.

The effect of partial soil sterilization on the incidence of disease of wheat due to H. sativum was investigated. One hundred-fifty grams of Alberta black soil were placed in 3 in. clay pots and subjected to various intensities of heat for varying periods of time. One third of the treated soils was immediately inoculated with H. sativum and sown to wheat. Another third was not inoculated but was sown to wheat. The remaining third was kept in plastic bags for four days before H. sativum was added and

wheat was sown. The results of this experiment have been compiled in Table IV.

TABLE IV. THE INFLUENCE OF RECOLONIZATION OF A PARTIALLY HEAT-STERILIZED SOIL ON H. SATIVUM AS INDICATED BY THE WHEAT SEEDLING TEST.

HEAT TREATMENT	H. SATIVUM*		H. SATIVUM**		CONTROL	
	PERCENT EMERGED	DISEASE RATE	PERCENT EMERGED	DISEASE RATE	PERCENT EMERGED	DISEASE RATE
None	75	0.1	75	0.1	70	0
Autoclaved momentarily to 15#	85	0.8	60	0.4	87	0
Steamed for 1 hr.	75	0.9	68	0.6	81	0
Autoclaved for 5 min.	83	1.1	55	1.0	93	0
Autoclaved for 15 min.	78	1.4	80	0.9	85	0
Autoclaved for 3 hr.	83	1.3	83	1.3	86	0

* H. sativum added immediately after heat treatment.

** H. sativum added 4 days after heat treatment.

The results shown in Table IV indicate that heat sterilization inactivated the natural soil microflora and permitted the establishment of the introduced pathogen. After four days, however, soils that were autoclaved momentarily to 15 lb. steam or steamed for one hour demonstrated an active microflora. The activity of this microflora was detected by its antagonistic or competitive effect on H. sativum which became less damaging to wheat seedlings than when grown in soil with little or no competing microflora. Soils heated for longer periods of time than indicated above were sterile.

The influence of formaldehyde on the natural flora of Alberta black soil was investigated. Commercial formaldehyde was used to prepare dilutions of 1:500, 1:300, 1:200 and 1:100. Counts of the microorganisms antagonistic to H. sativum were made by water dilutions of these soils on the triple agar described by Herr (1959). Dilutions prepared were 1:200,000.

The results in Table V show the emergence and disease rating of wheat seedlings, and the numbers of antagonists per gram of various formalin-treated soils.

TABLE V. THE EFFECT OF FORMALDEHYDE ON THE NATURE AND NUMBERS OF ANTAGONISTIC MICROORGANISMS IN NATURAL SOIL AS DETERMINED BY THE INCIDENCE OF DISEASE OF WHEAT SEEDLINGS DUE TO H. SATIVUM.

RATIO OF FORMALDEHYDE : WATER ADDED TO SOIL	DISEASE RATE	PERCENT EMERGED	NUMBERS OF ANTAGONISTIC MICROORGANISMS PER c.c. OF DILUTION
1;500	0.8	82	16
1:300	0.9	82	18
1:200	1.2	93	0.2
1:100	1.4	89	2.8
Control	1.5	72	Contaminated

The results in Table V indicate that an increase in the concentration of formaldehyde is reflected by an increase in the activity of the pathogen. This was correlated with the numbers of antagonists per gram of soil. An increase in the numbers of antagonists appears to lower the disease incidence. An increase in germination corresponds to a reduction in disease of the wheat seedlings.

(4) Survival of antagonists and H. sativum in sterile and natural soil

Katznelson (1940) reported a temporary increase followed by a rapid decrease in the growth of H. sativum when introduced into sterile soil. Katznelson (1940) also noted that growth of H. sativum decreased rapidly in sterile soil when introduced simultaneously with Streptomyces cellulosae and Streptomyces fradii.

The survival period of an antagonist, S. griseus, in natural and sterile soil was determined. S. griseus was selected because it produces the antibiotic actidione, which retains a high proportion of its activity for about nine days in natural soil and 50 days in sterile soil (Peterson 1952).

H. sativum inoculum was applied at two-day intervals to sterile and natural soil inoculated with S. griseus. At the end of the 14-day period all soils

were sown to wheat. The results are presented in Table VI.

TABLE VI. THE SURVIVAL OF S. GRISEUS OVER A PERIOD OF TIME IN NATURAL AND STERILIZED SOIL AS DETERMINED BY THE INCIDENCE OF DISEASE OF WHEAT SEEDLINGS DUE TO H. SATIVUM.

		DAYS AFTER WHICH H. SATIVUM WAS ADDED TO SOIL - S. GRISEUS MIXTURE																	
		0		2		4		6		8		10		12		14		Ck.	
SOIL	S. GRISEUS	E	D	E	D	E	D	E	D	E	D	E	D	E	D	E	D	E	D
Non-sterile	yes	71	1.1	63	2.3	70	1.8	82	1.6	80	1.8	60	2.8	50	3.0	73	2.1	93	0
	no	67	1.1	59	2.1	70	1.8	62	1.2	72	1.3	60	1.8	50	2.4	40	4.2	73	0
Sterile	yes	45	3.0	55	2.2	47	2.7	42	2.9	67	2.1	49	2.7	55	1.9	67	1.6	72	0
	no	59	2.9					20	4.4							67	4.0	87	0

E - percent emergence

D - disease incidence

H. sativum applied to sterile soil eight days or less before seeding wheat resulted in a disease rating of 4.4, while seedling damage was reduced to 2.9 when the pathogen was added 14 days or more before seeding. The activity of H. sativum diminished within two or three days after being added to natural soil.

S. griseus reduced the incidence of disease on wheat seedlings due to H. sativum in sterile and natural soil when the wheat was sown simultaneously with the pathogen. The antagonism of S. griseus to H. sativum was retained longer in sterile than in natural soil where its presence had no effect on H. sativum applied more than two days prior to sowing to wheat.

(5) Comparative Antagonistic Properties of Different Soils

There is scant reference in the literature on the comparative antagonistic properties of different soils to certain plant pathogens. Stover (1959) discussed the importance of physical factors, such as location within the profile, aeration, soil texture, moisture, pH and temperature, on fungal growth and survival in soil. He stated that heavy textured soils, which were usually neutral or alkaline in reaction,

were often richer in bacterial and actinomycete flora than the lighter textured ones. Fusarium wilts were generally favoured by light textured soils (Stover 1959). Jensen (1931) suggested that distinct fungal communities were associated with different soils. Warcup (1959) reviewed the literature pertaining to the microorganisms associated with different soils and stated that soils may contain definite fungal communities, but little was known of the factors which influence the distribution of the organisms.

Katznelson (1940) noted that all micro-organisms introduced into five different soils varying in organic matter and pH, decreased in number. Some, such as F. culmorum, disappeared entirely. He found that F. culmorum was most prominent in soils that were most active microbiologically, and suggested that this pathogen survived by virtue of its ability to utilize various decomposition products of materials, such as alfalfa straw.

The first experiment was designed to compare the antagonistic effect of the soil microflora from the three major Alberta soil groups, black, brown and grey wooded on several plant pathogens. The organic matter in the brown soil originated from wild grasses and, more recently, from cereal crops. The nature of the organic matter and the more severe

physical effect of the climate may influence the soil texture and thus its aeration and water-holding capacity. The black soils differ from the brown with respect to the type and amount of organic matter. Grey wooded soil is a degraded type in which much of the well-decomposed organic matter has been leached out.

The three soils were brought to optimum moisture-holding capacity for sowing wheat and inoculated singly with a cornmeal-soil inoculum of four pathogens: H. sativum, F. culmorum, F. graminearum and O. graminis. Samples of soil were removed at certain intervals of time and sown to wheat. Data of disease incidence and emergence are shown in Tables VII and VIII, respectively.

TABLE VII. THE SURVIVAL OF FOUR PATHOGENS IN THREE DIFFERENT SOILS OF ALBERTA AS DETERMINED BY THE EFFECT OF THE MICROFLORA ON THE INCIDENCE OF ROOT ROT OF WHEAT.

INTERVAL* OF SOWING AFTER INOCULATION	DISEASE INCIDENCE														
	O. GRAMINIS IN			H. SATIVUM IN			F. CULMORUM IN			F. GRAMINEARUM IN			CONTROL		
	G.W.	B.	BR.	G.W.	B.	BR.	G.W.	B.	BR.	G.W.	B.	BR.	G.W.	B.	BR.
0	1.4	2.6	2.8	2.5	1.2	2.3	5	5	5	5	4.6	4.2	0.3	0	0
1	0.8	0.3	1.2	1.6	1.3	1.8	4.6	4.6	3.2	4.8	4.6	3.8			
2	0.3	0.4	0.2	1.5	0.7	0.8	4.7	3.7	3.9	3.6	3.2	2.3			
4	0	0.1	0.4	1.5	0.4	0.6	4.8	1.9	2.5	2.8	1.9	0.9			
8	0.1	0	0	0.9	0.8	0.8	2.6	1.7	1.1	1.3	1.7	0.9	0	0	0

* Weeks

G.W. - Grey wooded soil

B. - Black soil

Br. - Brown soil

TABLE VIII. THE SURVIVAL OF FOUR PATHOGENS IN THREE DIFFERENT SOILS OF ALBERTA AS DETERMINED BY THEIR EFFECT ON WHEAT SEEDLING EMERGENCE.

INTERVAL* OF SOWING AFTER INOCULATION	EMERGENCE PERCENTAGE														
	O. GRAMINIS IN			H. SATIVUM IN			F. CULMORUM IN			F. GRAMINEARUM IN			CONTROL		
	G.W.	B.	BR.	G.W.	B.	BR.	G.W.	B.	BR.	G.W.	B.	BR.	G.W.	B.	BR.
0	80	95	73	72	83	72	5	0	3	0	20	29	63	87	87
1	79	92	79	79	87	75	13	15	52	12	15	40			
2	89	89	89	62	87	80	13	39	42	29	49	30			
4	83	89	83	63	85	80	19	75	63	49	65	83			
8	65	83	65	63	73	73	50	67	73	53	65	79	73	80	73

* Weeks

G.W. - Grey wooded soil

B. - Black soil

Br. - Brown soil

The results indicate that the extent of root rot of wheat seedlings caused by a fungal pathogen may vary in different soils. F. culmorum and F. graminearum appeared to be less severe on wheat growing in brown soil and most severe in grey wooded soil. O. graminis was generally more severe on wheat growing in brown soil than on wheat in grey wooded soil. When the wheat was sown simultaneously with the fungal inoculum, disease was almost twice as severe on wheat growing in grey wooded soil as in the other soils. The activity of O. graminis decreased the most rapidly in all soils. H. sativum survived for a longer period in grey wooded soil than in brown or black soil. F. culmorum survived best in the grey wooded soil. This pathogen caused some disease of wheat seedlings, even after eight weeks. The survival pattern of F. graminearum was similar to F. culmorum.

The percentage of germinated seedlings was not altered by O. graminis or H. sativum but was lowered in soils containing the two Fusaria.

The three soils were sampled for numbers of antagonists to F. culmorum by means of dilution plates described by Herr (1959). These results are recorded in Table IX.

TABLE IX. THE NUMBERS OF ANTAGONISTS INHIBITING *F. CULMORUM* *IN VITRO* PER GRAM OF THREE ALBERTA SOILS.

SOIL	ANTAGONISTS PER GRAM OF SOIL TO F. CULMORUM
Black	360,000
Brown	560,000
Grey wooded	200,000

Note: 10 replications per soil.

The reduced amount of disease of wheat due to *F. culmorum* in the brown soil may be associated with the greater number of antagonists in this soil.

A study of 17 soils representing the three soil types in Alberta was made to compare the numbers of microorganisms antagonistic to *H. sativum*. Seven of these soils were black, seven were grey wooded and three were brown. These soils were obtained from the Soils Department at this University where they had been amended with equal amounts of N and K. The results of this study are presented in Table X.

TABLE X. NUMBERS OF MICROORGANISMS ANTAGONISTIC TO H. SATIVUM PER GRAM OF THE THREE MAJOR SOILS OF ALBERTA.

SOIL	TOTAL NO. OF MICROORGANISMS	NO. OF ANTAGONISTS	PERCENT ANTAGONISTIC TO H. SATIVUM
Grey wooded	49,900,000	3,190,000	6.4
Black	55,700,000	6,940,000	12.5
Brown	94,800,000	6,520,000	6.9

Results summarized in Table X indicate that the proportion of antagonists to the number of microorganisms in the black soil is almost double that of the brown or grey wooded soils. It is interesting to note that the disease incidence of wheat due to H. sativum was almost half as severe on wheat growing in black soil as in the other soils. Data presented in Table XI lend support to this observation.

TABLE XI. THE DISEASE INCIDENCE OF WHEAT SEEDLINGS DUE TO H. SATIVUM IN THREE ALBERTA SOILS.

SOIL	DISEASE INCIDENCE	
	EXPERIMENT 1	EXPERIMENT 2
Grey wooded	2.7	2.5
Black	2.0	1.2
Brown	2.9	2.3

The toxic properties of various soils from Ghana and India were compared with those from Alberta; As only 300 -400 gm. of soil from Ghana and India were available, a small portion (2.2% by weight) of these soils was added to sterilized Alberta black soil in order to establish a soil microflora comparable to that of the contaminating soils. The behaviour of a few pathogens in the presence of the soil microflora from several soils in Ghana and Alberta was investigated.

Four hundred-fifty gm. of sterile black Alberta soil were amended with 10 gm. of non-sterile foreign soil and stored at 30°, 25°, 20°C., respectively. The mixtures were shaken daily for seven days before H. sativum inoculum was added. All soils were incubated for another six days before being sown to wheat and returned to appropriate temperatures for six more days. All pots were then kept at room temperature and the seedlings were harvested one week later. Table XII presents the data of the disease incidence and emergence of wheat seedlings.

TABLE XII. THE EFFECT OF TEMPERATURE ON THE DEVELOPMENT OF A MICROFLORA IN RECONTAMINATED STERILE SOIL AS DETERMINED BY ITS EFFECT ON THE INCIDENCE OF ROOT-ROT OF WHEAT DUE TO H. SATIVUM.

	H. SATIVUM						NO H. SATIVUM					
	DISEASE RATE			PERCENT EMERGENCE			DISEASE RATE			PERCENT EMERGENCE		
SOIL MIXTURE	30°C.	25°C.	20°C.	30°C.	25°C.	20°C.	30°C.	25°C.	20°C.	30°C.	25°C.	20°C.
1	1.2	0.9	1.5	82	83	73	0	0	0	93	80	93
2	1.7	0.9	0.9	79	97	89	0	0	0	100	93	80
3	3.3	2.0	1.8	58	57	57	0	0	0	93	67	73
4	2.5	2.4	1.1	47	70	47	0	0	0	60	77	37
5	2.2	4.3	3.6	79	33	37	0	0	0	90	100	77
6	0.5	1.4	1.5	90	73	67	0	0	0	83	84	80

Soil Mixtures: 1. Sterile & Ghana fallow
2. Sterile & Ghana forest
3. Sterile & Alta. fallow
4. Sterile & Greenhouse stock
5. Sterile
6. Greenhouse stock

Sterile soil was obtained from the greenhouse stock supply of black soil. The soil microflora of soils numbers 4 and 6 should theoretically be similar.

It appeared that a representative microflora was established in recontaminated soils at the lower but not the higher temperatures. The microflora from different soils differed in the degree of antagonism to H. sativum. Microflora from the Ghanaian soils maintained at 30°C. and 25°C. was considerably more antagonistic to H. sativum than microflora from local soils, whereas at lower temperatures the microflora from the local soils was as effective in inhibiting H. sativum as that from Ghanaian soils.

Soils and treatments, listed in Table XIII, were used to show the effects of their microflora on the incidence of disease caused by F. culmorum and F. graminearum. The soils were prepared and amended as for the previous experiment.

The incidence of disease of wheat due to F. graminearum was effectively reduced in the two Alberta soils, suggesting the rapid recolonization by the soil microflora. The incidence of disease due to F. culmorum was not reduced as markedly in the two Alberta soils as in the case of F. graminearum.

TABLE XIII. THE EFFECT OF THE MICROFLORA FROM VARIOUS SOILS, WHEN INTRODUCED INTO A STERILE BLACK ALBERTA SOIL, ON THE INCIDENCE OF DISEASE AND PERCENT EMERGENCE OF WHEAT SEEDLINGS DUE TO F. CULMORUM AND F. GRAMINEARUM.

	F. GRAMINEARUM		F. CULMORUM		CONTROL	
SOIL MIXTURE*	PERCENT EMERGED	DISEASE RATE	PERCENT EMERGED	DISEASE RATE	PERCENT EMERGED	DISEASE RATE
Alberta black	80	0.8	75	1.4	97	0
Taber virgin grass	77	0.9	78	1.3	90	0
Ghana forest	62	3.0	50	3.5	87	0
No contaminating soil	52	3.9	57	2.7	77	0
Non-sterile Alberta black	83	0.9	42	3.1	77	0

* Sterile soil plus contaminating soil

Sterile Alberta black soil recontaminated with the same non-sterilized soil theoretically has the same microflora as the non-sterile Alberta black soil.

(6) The use of an artificial soil as a medium for the multiplication of soil microflora

A non-nutritive cellulose powder (alphacell) was used as a base in which foreign soils were incorporated. Such a base would eliminate the possible existence of toxic products which might influence the establishment of a typical microflora in steam-sterilized soil. In a similar experiment, greenhouse stock soil was used in place of alphacell. The wheat seedlings were scored for disease and emergence two weeks after seeding, and results were recorded in Table XIV.

TABLE XIV. THE EFFECT OF THE MICROFLORA FROM VARIOUS SOILS, WHEN INTRODUCED INTO A STERILE BLACK ALBERTA SOIL AND STERILE ALPHACELL, ON THE INCIDENCE OF DISEASE AND PERCENT EMERGENCE OF WHEAT SEEDLINGS DUE TO H. SATIVUM.

	STERILE ALPHACELL				STERILE ALBERTA BLACK SOIL			
	H. SATIVUM		CONTROL		H. SATIVUM		CONTROL	
CONTAMINATING SOIL*	PERCENT EMERGED	DISEASE RATE	PERCENT EMERGED	DISEASE RATE	PERCENT EMERGED	DISEASE RATE	PERCENT EMERGED	DISEASE RATE
1	67	1.9	72	0.2	53	1.1	72	0
2	60	1.2	80	0.3	72	0.4	47	0
3	72	0.9	80	0.1	60	0.2	34	0
4	80	0.4	72	0	64	0.4	67	0
5	34	4.4	72	0.4	47	3.9	72	0

* Contaminating Soil:
1. Ghana forest
2. Alberta black - obtained from the greenhouse stock of black soil
3. Campus soil (fallow)
4. Campus soil (fallow) - soil was frozen at -20°C. for 8 months prior to its use
5. None added

The root rot of wheat caused by H. sativum was slightly more severe in alphacell than in soil. This was compensated for by a slight overall increase in germination of wheat in alphacell. Some discolouration was noted on the roots and basal portion of the wheat seedlings growing in alphacell without H. sativum, whereas the soil without pathogen yielded clean seedlings. It is possible that toxic effects in the recontaminated sterilized soil controlled seed-borne microorganisms which could be mildly parasitic under conditions that may have occurred in alphacell.

Another experiment was conducted with soils from India, grey wooded, black and brown soils from Alberta, Ghana forest and fallow soils and greenhouse stock soil. The sterile base soil was Alberta black soil, steam-sterilized for three hours at 20 lb. pressure. The incidence of disease caused by H. sativum and F. culmorum in the presence of the microflora from the above soils was determined by the wheat seedling tests and the results were recorded in Table XV.

TABLE XV. THE EFFECT OF THE MICROFLORA FROM VARIOUS SOILS, WHEN INTRODUCED INTO A STERILE BLACK ALBERTA SOIL, ON THE INCIDENCE OF DISEASE AND PERCENT EMERGENCE OF WHEAT SEEDLINGS DUE TO H. SATIVUM AND F. CULMORUM.

	H. SATIVUM		F. CULMORUM		CONTROL	
CONTAMINATING SOIL	PERCENT EMERGED	DISEASE RATE	PERCENT EMERGED	DISEASE RATE	PERCENT EMERGED	DISEASE RATE
Alberta brown	79	1.4	15	4.5	80	0.3
Alberta black *	71	1.7	35	3.8	93	0
Alberta grey wooded	67	2.3	39	3.1	93	0
Ghana forest	70	2.8	7	4.8	100	0
Ghana fallow	70	2.0	7	4.8	100	0.1
India	73	1.7	17	3.5	100	0.1
1	60	3.2	0	5	93	0
2*	67	1.9	29	3.5	80	0

1. Sterile Alberta black soil

2. Non-sterile Alberta black soil

* These soils theoretically have a similar microflora.

The incidence of disease on wheat seedlings caused by F. culmorum appeared to be more severe in brown soil than in black or grey wooded soils. H. sativum was more severe on wheat in grey wooded soil than in the other two Alberta soils. These results are similar to those obtained in other experiments where the antagonistic action of the three major Alberta soils was compared.

The soils from Ghana appeared to possess little antagonism towards H. sativum and F. culmorum. The reason for the greatly reduced antagonism to H. sativum at this time may be that the microflora of the soil sample had altered over the time. The soils were stored in a refrigerator to reduce microbial activity to a minimum. One and a half years after they were collected, the Ghana soils yielded a predominance of pink fungal colonies in dilution plates. This was evidence of their alteration because these colonies were rare in freshly collected soil.

It is interesting to note that the microflora in the recontaminated sterile greenhouse stock soil possessed similar antagonistic activity towards H. sativum and F. culmorum as did the original soils.

II. THE EFFECT OF SOIL FUMIGANTS, HERBICIDES AND FUNGICIDES ON THE ANTAGONISTIC ACTION OF THE NATURAL SOIL MICROFLORA TOWARDS SOME PATHOGENS.

LITERATURE REVIEW

The microbial ecology of the soil may be greatly altered by some soil amendments. Interest is focused on that sphere of the environment that will selectively enhance the activity of the antagonists. The effects of some amendments may persist in the soil and may influence the natural microflora as well as the invading pathogen. The more volatile amendment may alter the soil microflora, but it may have little direct effect on the pathogens introduced subsequently.

Amendments may influence the soil microflora in a number of different ways. These include alteration of soil pH, moisture holding capacity, aeration, the production of toxic substances, the encouragement of specific microorganisms and temperature changes. Amendments have been shown to stimulate the germination of the more resistant resting stage of a pathogen in the soil. Garrett (1956) discussed the antagonistic product, carbon dioxide, which is formed as a result of greatly increased microbial growth in amended soils.

The effects of pathogens on a crop can be reduced by rotations. The disease is minimized possibly because of a reduction of inoculum potential and an alteration in soil microflora which may be more antagonistic to the pathogen. Voronkevich et al. (1958) applied certain mineral and organic fertilizers to podsols and recorded a 5 to 26% increase in actinomycetes antagonistic to Erwinia carotovora and Bacterium aroideae. Sanford (1926), and Millard and Taylor (1927) suggested the possibility of biological control of potato scab, caused by Streptomyces scabies, by incorporating green manure into the soil. This apparently caused the saprophytic microorganisms to multiply and thus inhibited the pathogen.

Rouatt and Atkinson (1950) incorporated certain cover crops and examined the microbiological balance in potato scab-infested soils. Soybeans incorporated into soil greatly reduced the incidence of scab on potatoes. Soybean amended soils showed an increase in the population of actinomycetes, bacteria and fungi, while rye failed to increase the numbers of actinomycetes and, when used as a cover crop, failed to reduce scab disease incidence. It was also noted that the soybean material stimulated amino acid-requiring bacteria, while those requiring more complex compounds were suppressed.

Grossbard (1952, 1953) observed antibiotic production by Penicillium patulum in soils amended with a variety of substances. It was noted that carbon compounds, such as glucose, induced an increased antibiotic activity when compared to the same soils unamended.

Many forms of organic matter have been used as soil supplements, some of which effectively reduced specific pathogens. Decomposition of organic matter is the result of sequential colonization by a series of microorganisms, each succeeding group utilizing the metabolic byproducts of its predecessors. The soil microflora responsible for soil antagonism would, therefore, be expected to vary over a period of time. Many workers have reported an increase in total numbers of actinomycetes, but no increase in the percent of antagonistic actinomycetes as a result of amending the soil. It appears that the results of the use of amendments in the form of plant residues to encourage an antagonistic microflora towards certain pathogens is largely a matter of chance.

Due to the complex nature and dynamic state of a biotic system, it appears that simpler compounds might have a more direct effect on the soil microflora. Butler (1953) noted that ample nitrogen prolonged the survival of O. graminis and Curvularia ramosa but shortened that of H. sativum. He suggested that H.

sativum was suppressed by the straw-inhabiting competitors which were encouraged by nitrates. The saprophytic-colonizing ability of H. sativum and O. graminis was restricted in the presence of the soil microflora. Experiments reported here indicate that H. sativum persisted longer in the soil, possibly on infested organic matter thus increasing the inoculum potential.

Broadfoot and Tyner (1937) studied the relation of phosphorus, potassium, nitrogen, and calcium nutrition to the foot and root-rot diseases of wheat caused by H. sativum. They found that a lack of any of the above elements resulted in undernourished plants which were predisposed to attack by H. sativum.

Chemical substances have been used for some time to achieve a partial sterilization of the soil. Katznelson and Richardson (1943) compared the recolonizing microflora of soils recovering from the sterilizing effects of formaldehyde, chloropicrin and steam. They noted little damage to roots of tomato grown in soil treated with formaldehyde as compared with those grown in control soil. Numbers of fungi in formalin-treated soil increased three to four times as compared to those in natural soil. Bacteria increased three to five times, while the numbers of actinomycetes remained unchanged. The predominant fungus on the

roots of tomato plants grown in formalin-treated soil was found to be Trichoderma sp. Singh and Crump (1953) also reported an increase in bacterial numbers in soils treated with formalin.

The many weed-killing chemicals that are widely used at present might have some effect on the soil microflora. The effects of some of the more commonly used chemicals, such as 2,4-D, have been investigated. Such chemicals may exert their influence directly or indirectly through their breakdown products. Bell (1957) described a bacterium that caused the breakdown of 2,4-D. Jones (1952) found that the numbers of bacteria in soil were increased by large doses of 2,4-D and DDT, and that these compounds also affected nitrification at application rates used in practice. Warren et al. (1951) reported the dominance of an actinomycete in the soil microflora of a greenhouse soil treated with 2,4-D. This would suggest a greater tolerance to 2,4-D by actinomycetes than by bacteria or fungi. Yu-tien Hsia and Christensen (1951) found that 2,4-D increased the susceptibility of wheat to H. sativum. 2,4-D in culture inhibited H. sativum and resulted in some variation in the colony.

EXPERIMENTAL RESULTS

The effect of a number of chemical substances and organic compounds on microflora of greenhouse black stock soil was investigated. Results in Table XVI record the incidence of disease of 3-week old wheat seedlings caused by H. sativum.

TABLE XVI. THE INFLUENCE OF THE MICROFLORA IN A BLACK SOIL AMENDED WITH SOME AGRICULTURAL CHEMICALS AND GLUCOSE, ON THE INCIDENCE OF ROOT-ROT CAUSED BY H. SATIVUM OF WHEAT SEEDLINGS.

SOIL AMENDMENT	H. SATIVUM		NO H. SATIVUM	
	PERCENT EMERGED	DISEASE RATE	PERCENT EMERGED	DISEASE RATE
Formaldehyde 2%	68	0.5	92	0
2,4-D 100 ppm.	39	1.3	37	0.1
2,4-D 1000 ppm.	10	2.2	25	0
Arasan 0.1 gm./1200 gm. soil	67	2.1	82	0.1
D-113 @ 50 lb./acre	0	5.0	20	0.7
Bayer 2555 - 50 ppm.	75	1.3	83	0
Glucose 1%	77	1.2	87	0
Bayer 2555 - 500 ppm.	75	2.8	85	0.2
Control	50	1.8	68	0

All soil amendments, with the exception of the formaldehyde treatment, failed to reduce the root rot of wheat caused by H. sativum. Some amendments may have weakened and predisposed the wheat seedlings to preemergence attack by H. sativum. The emergence of wheat in the formalin-treated soil was 92%, while the same treatment including the pathogen reduced emergence to 68% with little evidence of disease on the seedlings.

Another experiment was conducted using some agricultural chemicals, plant residue and glucose as amendments in black soil. The results of this experiment are shown in Table XVII.

TABLE XVII. THE INFLUENCE OF THE MICROFLORA IN A BLACK SOIL AMENDED WITH SOME AGRICULTURAL CHEMICALS, GLUCOSE AND PLANT PRODUCTS, ON THE INCIDENCE OF ROOT-ROT CAUSED BY H. SATIVUM OF WHEAT SEEDLINGS.

SOIL AMENDMENT	H. SATIVUM		NO H. SATIVUM	
	PERCENT EMERGED	DISEASE RATE	PERCENT EMERGED	DISEASE RATE
Formaldehyde 5%	93	1.9	97	1.2
2,4-D 50 ppm.	63	0.9	55	0
D-113 100 ppm.	90	0.7	96	0
D-113 50 ppm.	87	1.0	87	0.1
Soya Flour 1%	75	0.4	96	0
Wheat Straw 1%	79	0.4	98	0
Avadex 1/2 lb./acre	79	1.1	89	0
Carbyne 1 lb./acre	70	0.7	96	0
Glucose 0.5%	90	1.1	93	0
Control	90	1.1	85	0

Soya flour and wheat straw considerably reduced the incidence of disease of wheat due to H. sativum. Formalin (5%) was too concentrated and thus weakened and predisposed the seedlings to attack by the pathogen or weak parasites and saprophytes in the soil. Wheat seedlings grown in the formalin-(5%) treated soil without H. sativum were scored at a disease rating of 1.2 which was only slightly less than the same treatment with H. sativum.

(1) Comparison of antagonistic properties of soils with the numbers of antagonistic microorganisms

The numbers of microorganisms antagonistic to some fungal pathogens was recorded in the amended soils. Dilution plates of such soils were prepared as described by Herr (1959). The results shown in Table XVIII demonstrate the effects of various amendments on the total numbers of microorganisms and percentage of microorganisms antagonistic to H. sativum. The numbers of microorganisms are recorded for one gram of air-dry soil.

TABLE XVIII. THE TOTAL NUMBERS OF MICROORGANISMS AND THE PERCENT OF MICROORGANISMS ANTAGONISTIC TO H. SATIVUM PRESENT PER GRAM OF SOIL TREATED WITH VARIOUS AGRICULTURAL CHEMICALS AND PLANT PRODUCTS.

SOIL AMENDMENT	pH AT TIME OF SAMPLING	PERCENT ANTAGONISTS	TOTAL NUMBER OF MICROORGANISMS PER GRAM
Formaldehyde 5%	5.6	25	150,000
2,4-D (50 ppm.)	5.8	19.4	5,250,000
D-113 (100 ppm.)	5.9	14.1	11,700,000
D-113 (50 ppm.)	6.0	11.2	12,900,000
Soya Flour 1%	6.5	*	332,500,000
Wheat Straw 1%	5.6	*	265,000,000
Avadex 1/2 lb./acre	5.7	11.5	13,150,000
Carbyne 1 lb./acre	5.9	17.5	8,350,000
Glucose 0.5%	5.6	*	31,300,000
Control	6.0	7.3	16,950,000

Disease incidence of wheat seedlings as recorded in Table XVII is associated with the numbers of antagonistic microorganisms as shown in Table XVIII. Since the dilution plates prepared from soils amended with soya flour, wheat straw and glucose were too crowded with soil microorganisms, little growth of the pathogen occurred. All other soil treatments caused a reduction in the total numbers of microorganisms, but increased slightly the percentage of antagonists. Soil amendments appeared to have little effect on the pH of the soil.

The microflora of amended Alberta soils was studied. Black, brown and grey wooded soils were amended with 1% (by weight) soya flour and 1% (by weight) alfalfa plant (powdered) and incubated for 23 days. Dilution plates at the rate of 1 gm. of soil to 10,000,000,000 ml. of water were poured and antagonistic microorganisms were detected by clear zones indicating a lack of growth of H. sativum around certain antagonistic microorganisms. Control soil was diluted 1 to 100,000. All soils were divided into two portions, one lot receiving 3.3% (by weight) of a 5% cornmeal-soil-H. sativum inoculum, and the other receiving the same mixture without the pathogen. The plants were counted and rated for disease after 21 days. The results of these investigations are presented in Table XIX.

TABLE XIX. THE INFLUENCE OF THE MICROFLORA OF BLACK, GREY WOODED AND BROWN SOILS AMENDED WITH SOME PLANT PRODUCTS, ON THE EMERGENCE AND INCIDENCE OF ROOT-ROT CAUSED BY H. SATIVUM OF WHEAT SEEDLINGS, NUMBERS OF MICROORGANISMS, AND NUMBERS OF MICROORGANISMS ANTAGONISTIC TO H. SATIVUM.

SOIL & AMENDMENT	H. SATIVUM		NO H. SATIVUM		TOTAL MICROORGANISMS PER GM. OF SOIL	ANTAGONISTIC MICROORGANISMS PER GRAM
	PERCENT EMERGED	DISEASE RATE	PERCENT EMERGED	DISEASE RATE		
G. wooded	60	2.7	70	0	2.13 million	850,000
G. wooded & alfalfa 1%	59	1.0	57	0	27.18 billion	950,000
G. wooded & soya flour 1%	60	1.5	75	0	16.7 billion	730,000
Black*	70	2.0	75	0	4.63 million	1,930,000
Black* & alfalfa 1%	65	1.2	67	0	40.0 billion	1,110,000
Black* & soya flour 1%	60	2.1	83	0	16.44 billion	1,720,000
Brown	49	2.9	70	0	1.64 million	630,000
Brown & alfalfa 1%	55	2.0	79	0	1.99 billion	620,000
Brown & soya flour 1%	50	2.0	65	0	12.08 billion	3,470,000

* This soil, which remained outdoors all winter, was from the same source as that used in previous experiments.

Root rot of wheat seedlings, caused by H. sativum, in the three soils was reduced by one-third to one-half by the addition of organic matter. Some plant products, particularly alfalfa meal, appeared to be most effective in the grey wooded soil in reducing the incidence of root rot of wheat seedlings. Alfalfa meal reduced root rot by about the same amount in the brown and black soils.

There is a closer relationship between the total numbers of microorganisms and disease rating of seedlings than between the numbers of antagonists and the disease rating. The numbers of antagonists per gram of air-dry soil in amended soils may be higher than indicated. It was expected that the high dilutions would produce colonies sufficiently sparse to be counted, but indications were that more dilute solutions should have been used. The large number of colonies on such plates may have given greater opportunity for antagonistic interactions, and thus resulted in unreliable counts.

An experiment was designed to determine the amount of root rot caused by H. sativum on wheat in a black soil amended with soya flour, starch, peptone, phenol or alphacell. The counts representing the

numbers of soil microorganisms were the average of six replications. The results of this experiment are recorded in Table XX.

TABLE XX. THE EFFECT OF SOME AMENDMENTS ON THE SOIL MICROFLORA OF BLACK SOIL AS DETERMINED BY THE INCIDENCE OF ROOT-ROT, CAUSED BY H. SATIVUM, OF WHEAT, AND ON THE NUMBERS OF MICROORGANISMS ANTAGONISTIC TO H. SATIVUM.

AMENDMENT	H. SATIVUM		NO H. SATIVUM		TOTAL NUMBERS OF MICROORGANISMS
	PERCENT EMERGED	DISEASE RATE	PERCENT EMERGED	DISEASE RATE	
Soya Flour 1%	87	0.3	67	0	6.0 billion
Starch 1%	73	1.2	73	0	8.81 million
Peptone 1%	87	1.0	73	1.0	
Phenol 0.5%	0		0		
Alphacell 1%	86	0.5	73	0	10.86 million
Control	71	2.2	80	0	6.56 million

The results summarized in Table XX show that root rot of wheat caused by H. sativum was reduced by all amendments except phenol, which was toxic to the wheat seedlings. Disease incidence was reduced by at least one-half. Peptone, at the rate used, caused necrosis of the roots of the wheat seedlings similar to damage caused by H. sativum. There was some association between total microorganism count and disease incidence. The soils containing the greatest numbers of microorganisms produced fewer diseased plants.

The effect of amendments on certain known antagonists in sterile and non-sterilized black soil was also studied. The antagonists used include one actinomycete No. 284, B. subtilis A-32 and T. viride. The organic amendments were 5% (by weight) glucose, 5% (by weight) green manure (chopped wheat plants at the heading stage), and a combination of the two. The antagonist and the pathogen were added to the soil as spore suspensions in sterile distilled water. The results of this experiment are recorded in Table XXI.

TABLE XXI. THE EFFECT OF GLUCOSE AND GREEN MANURE IN STERILE AND NON-STERILE SOIL ON THE EMERGENCE AND INCIDENCE OF DISEASE OF WHEAT SEEDLINGS DUE TO H. SATIVUM.

SOIL TREATMENT		ANTAGONIST	H. SATIVUM		NO H. SATIVUM	
			PERCENT EMERGED	DISEASE RATE	PERCENT EMERGED	DISEASE RATE
Soil	Sterile	Act. 284	96	0	100	0
		B. subtilis	96	0	87	0
		T. viride	93	0	83	0
		Control	49	4.0	90	0
	Non-Sterile	Act. 284	38	1.8	77	0
		B. subtilis	62	1.0	80	0.1
		T. viride	47	3.0	93	0
		Control	60	1.1	67	0

TABLE XXI (CONTINUED)

SOIL TREATMENT		ANTAGONIST	H. SATIVUM PERCENT EMERGED	H. SATIVUM DISEASE RATE	NO H. SATIVUM PERCENT EMERGED	NO H. SATIVUM DISEASE RATE
Soil & 5% Glucose	Sterile	Act. 284	22	0	3	0
		B. subtilis	49	0	10	0
		T. viride	38	0.2	33	0
		Control	60	0.1	10	0
	Non-sterile	Act. 284	16	3.1	77	0
		B. subtilis	0	5	67	0.1
		T. viride	0	5	23	0
		Control	69	0.1	40	0

TABLE XXI (CONTINUED)

SOIL TREATMENT		ANTAGONIST	H. SATIVUM PERCENT EMERGED	H. SATIVUM DISEASE RATE	NO H. SATIVUM PERCENT EMERGED	NO H. SATIVUM DISEASE RATE
Soil & 5% Green Manure	Sterile	Act. 284	36	1.9	70	0
		B. subtilis	87	0.3	90	0
		T. viride	96	0.1	80	0
		Control	93	0.5	87	0.3
	Non-sterile	Act. 284	58	0.3	7	0
		B. subtilis	51	0.7	57	0.1
		T. viride	71	0.2	60	0.1
		Control	60	1.0	67	0

TABLE XXI (CONTINUED)

SOIL TREATMENT		ANTAGONIST	H. SATIVUM PERCENT EMERGED	H. SATIVUM DISEASE RATE	NO H. SATIVUM PERCENT EMERGED	NO H. SATIVUM DISEASE RATE
Soil & 5% Glucose & 5% Green Manure	Sterile	Act. 284	4	0.1	3	0
		B. subtilis	16	0.1	7	0
		T. viride	71	0.2	43	0
		Control	20	0.4	10	0
	Non-sterile	Act. 284	7	3.1	53	0.1
		B. subtilis	13	1.6	17	0
		T. viride	9	0	7	0
		Control	7	0	10	0

The results shown in Table XXI indicate that the antagonists in sterile soil may effectively reduce the incidence of root rot of wheat due to H. sativum. This effect was not noted in natural soil; possibly because the inoculum which was applied as ungerminated spores was more susceptible than pre-germinated spores and hyphae to antagonistic products in the natural soil.

The use of sugar alone, or with green manure, depressed emergence and increased the disease incidence of wheat seedlings in natural soil. This effect was less marked in sterile soil.

Green manure, which was non-sterile, introduced into the sterile soil many microorganisms that reduced the amount of root rot of wheat due to H. sativum. Root rot of wheat seedlings was more severe in non-sterile soil amended with green manure.

The combined effect of sugar and green manure as a soil amendment reduced the germination of wheat, both in the presence and absence of H. sativum. This may be due to the soil microflora or to toxic effects of sugar and soil chemical interactions.

DISCUSSION AND CONCLUSIONS

(1) The presence of antagonistic microorganisms in the soil

Antagonism to some pathogens in natural soil is due partly to the activity of soil microorganisms. Sterile alphacell or sterile soil, when inoculated with a pinch of natural soil, was colonized rapidly by the soil microflora. H. sativum, introduced subsequently, failed to develop to the same extent as in sterile alphacell or soil.

Actinomycetes appeared to play a major role in minimizing the effects of the soil-borne plant pathogens. This fact was supported by studies of antagonism in natural soil and in soil dilution plates on Czapek's agar. References to support these observations have been noted. (Stessel, Leben and Keitt 1953, Schatz and Hazen 1948, and Landerkin 1950).

Some workers have noted the importance of other groups of microorganisms as antagonists (Luke and Connel 1954, Morton and Stroube 1955).

The media and methods employed in detecting antagonistic microorganisms may differ greatly. Czapek's medium, used in these investigations, favoured

the development of actinomycetes and tended to place undue emphasis on them. The morphological features of actinomycetes, however, may be to their advantage in extensive colonization of their environment. Spreading mycelia increase areas of infection, while the prolific production of spores ensures their wide distribution. The ubiquity of actinomycetes is evident by their odour upon turning fresh sod.

Fungi may be important as antagonists in certain environments, such as those encountered in sterilized soils which are being recolonized. Fresh organic matter, which is a good substratum for various fungi, may cause them to develop so abundantly as to inhibit some pathogens. Such environmental conditions are generally limited to the surface layer of soil. Other fungi are known to produce antibiotics which may inhibit the activity of fungal pathogens. Garrett (1956) noted that as early as 1897 Duchesne reported that some Penicillia inhibited the development of certain bacteria. More recent work with this group of fungi by Grossbard (1953) has indicated that the antagonistic property is due to antibiotics. Other antagonistic fungi, of which T. viride is well-known, produce antibiotics (Weindling 1936). Ludwig (1939) noted the importance of this fungus in recolonizing sterile soil. Antagonists have been reported from

most groups of the fungi with the exception of the Phycomycetes (Waksman 1947).

Results reported in these investigations clearly indicate the antagonistic effects of some actinomycetes, a fungus and a bacterium towards several plant pathogens in sterile soil. These antagonists are saprophytic in nature and can compete favourably with the plant pathogen which survives best in the presence of a suitable host. Many plant pathogens may compete more effectively with antagonistic microorganisms by developing a resistant stage or by utilizing a wider range of organic matter. Evidence was presented here that F. graminearum and F. culmorum could persist in natural soil in the absence of a living host plant for at least eight weeks. Garrett (1956) suggested that the Fusaria were able to survive longer in soil than some other fungi as they were better adapted to a saprophytic existence.

The antagonism of S. griseus to H. sativum dissipated within two days in natural soil and within eight days in sterile soil. The sterile soil which was exposed to the air in the greenhouse probably became colonized by air-borne microorganisms which competed effectively with the actinomycete.

The antagonism of microorganisms to pathogens, and the activity of the pathogens on their host plants were less severe in the non-sterile than in the sterile soil.

Competition with the natural soil microflora for space and food, quickly reduced the numbers of the introduced antagonists and thus lessened their effects. Henry (1931) noted the reduced activity of H. sativum in recontaminated and non-sterile soils as compared to sterile soil.

Not all actinomycetes are equally effective as antagonists to a pathogen in soil though they may have displayed equal degrees of antagonism in vitro. Some actinomycetes may produce antibiotics under certain conditions or in the presence of certain substances (Wright 1956). S. griseus and actinomycete No. 284 were more effective in reducing the activity of H. sativum in sterile soil than actinomycetes Nos. 380 and 453, even though both groups showed the same degree of antagonism towards H. sativum on agar. Thayson (1950) doubted if microorganisms that produce antibiotics in vitro, did so in the soil.

Due to the complex nature of the soil microflora and the various metabolic by-products of the members, it is possible that interactions of these products may inactivate some antibiotics. Antibiotics also may be decomposed to simpler compounds by certain microorganisms in the soil, and some also may be adsorbed by certain fractions in the soil as summarized by Jefferys (1952).

Unavailability of food through competition may be one of the reasons why disease severity due to fungi, introduced as spores into recontaminated or natural soil, is quickly reduced. H. sativum, when applied to the soil as a 5% cornmeal-soil mixture, was more virulent or persistent in the soil than when applied as a spore suspension in water. There is a greater inoculum potential in the case of pre-germinated spores and hyphae growing on organic matter (corn meal). Inoculum applied as ungerminated conidia was effective only if placed adjacent to a suitable infection court. It appears that some stimulation was necessary for the development of the fungus from the spores. Such soil amendments as soya flour will cause certain spores to germinate in natural soil (Chinn 1953). If the germinating spores fail to make contact with a suitable substratum, they may perish due to toxic factors in the soils. Such toxic factors may be of biological origin (Neilson-Jones 1941).

(2) Recolonization of partially-sterilized soils

Very small amounts of contaminating soil could recolonize a sterile soil if the introduced microflora were allowed to multiply for five days. Ludwig and Henry (1943) studied the microflora quantitatively and qualitatively at various periods

of time after recontamination of sterile soil. They noted the reduced activity of O. graminis in recontaminated sterile soil. They found that all recontaminated soil contained more microorganisms than natural soil and thus explained the greater reduction in the effect of a pathogen on its host in recontaminated soil.

Soils used in recontamination studies in these investigations were heated. It was found that autoclaving soils for any period of time longer than being held momentarily at 15 lb. steam pressure inactivated the soil microflora. Less severe heat, such as produced by steaming the soil, considerably reduced the numbers of microorganisms. Warcup (1951a) noted that the microorganisms surviving partial sterilization of soil by heat were often certain Ascomycetes. The remaining microorganisms multiplied within several days to reduce effectively the activity of H. sativum on wheat.

Steaming would be expected to alter the soil chemically and physically. Malowany and Newton (1947) noted that water-soluble phosphate was greatly increased and water soluble sulphate was somewhat increased in three Alberta soils by steam sterilization. Phosphorus was increased by one-third in the black soils, but not so in the brown or grey wooded. Ammonia accumulated in black and brown soils for four to six weeks. Many

ammonia-producing bacteria are spore-forming, which may explain their early appearance after the soil is treated with steam. These workers stated that nitrification was suppressed for about six weeks in sterilized recontaminated soils, after which it became more active than in natural soil.

Certain physical properties, such as capillarity and water-holding capacity, were affected by steam sterilization. These physical factors may have some influence on the type of recolonizing microflora.

Partial soil sterilization as may be practised in commercial greenhouses could be dangerous particularly when there is the possibility of introducing pathogens.

(3) Antagonistic properties of different soils

The antagonistic properties of several geographically isolated soils were examined. Since organic matter is an important constituent of the soil, it may influence the amount and type of microorganisms present. Black soils from Alberta contained more organic matter than the other soils used and consequently supported more microorganisms antagonistic to the pathogens used in these investigations. (See Appendix I)

Soils from Ghana appeared to contain microflora more antagonistic to several locally-obtained pathogens than did local soils. This was particularly evident at the higher soil temperatures which were comparable with those normal for Ghana. The apparent antagonism to H. sativum in this soil at 30°C. may have been complicated by the direct effect of temperature on the pathogen.

Recontamination of sterile soil with the same natural soil does not establish a microflora similar to the natural soil at 30° and 25°C. but does so at 20°C. Henry (1932) noted that O. graminis was virulent towards wheat in sterile and non-sterile soil at low temperatures but was less virulent at higher temperatures in non-sterile soil. This suggested that at the higher temperatures the soil microflora was more antagonistic to O. graminis. Temperature, therefore, is an environmental factor that may play an important part in determining the type of microflora.

(4) The influence of amendments on the antagonistic soil microflora

Amendments with a low carbon to nitrogen ratio, such as alfalfa meal, green wheat plants and soya flour, encouraged a soil microflora antagonistic

to H. sativum. The decrease in activity of the pathogen, in some cases corresponded to an increase in the proportion of microorganisms antagonistic to H. sativum. These microorganisms were predominantly actinomycetes and some bacteria. Total numbers of microorganisms, however, had a greater effect on the activity of the pathogen. Soya flour and wheat straw, particularly in the black and grey wooded soil, induced a great increase in numbers of microorganisms. Competition for food and space might effectively reduce the amount of H. sativum. The more abundant organic matter available in the black soil may be responsible for the larger populations of microorganisms. Rouatt and Atkinson (1950) noted that soybeans as a cover crop reduced the incidence of scab of the succeeding crop of potatoes. Such soils were shown to have an increased population of actinomycetes, bacteria and fungi. As the soil microflora may be influenced by the carbon to nitrogen ratio of the amendment, glucose with a high carbon ratio and green wheat straw with a high nitrogen ratio were incorporated into soils that were inoculated with H. sativum and sown with wheat. The greenmanure effectively reduced the activity of H. sativum on wheat plants, while glucose had no effect. There was some indication of toxicity to wheat seedlings grown in glucose-amended soils, possibly predisposing them to disease. Snyder

et al. (1959) found that the carbon to nitrogen ratio influenced the degree of root-rot of beans caused by Fusarium solani, Rhizoctonia solani and Thielaviopsis basicola. Green barley straw increased disease while mature straw reduced it. The latter effect could be negated by adding nitrogen. Tyner (1940) suggested that the carbon to nitrogen ratio of the various grain straws had less effect on disease development than did the chemical nature of the straw.

Aside from increasing the soil microflora, the plant residues may have stimulated the spores of the pathogen to germinate, thus making them more susceptible to the antibiotic products in the soil. Boosalis (1960) noted that the spores of H. sativum failed to germinate in natural soil treated with water, whereas he observed conidial germination in natural soils amended with dextrose, potato broth or both.

Amendments of non-plant origin, such as some agricultural chemicals, usually reduced the numbers of soil microorganisms indicating toxicity. Accordingly their use failed to reduce the virulence of H. sativum. Formaldehyde, however, was an exception. Soils treated with this chemical may have been recolonized by such antagonistic microorganisms as T. viride (Warcup 1951). Many of the chemical substances may be decomposed by certain microorganisms to simpler compounds. Bell (1957) described a soil bacterium responsible for the decomposition of 2,4-Dichloro-

phenoxyacetic acid. Soils treated with this chemical would, therefore, encourage a greater proportion of these microorganisms. Since the immediate breakdown products of such chemicals are less complex than those of plant materials, it may be expected that their effects would be less persistent. For this reason, plant residues would be more useful in biological control of plant pathogens. Early stages of plant decomposition would inhibit plant pathogens through competition and the latter stages of decomposition through metabolic by-products. The use of certain chemicals might be warranted if it were known that microorganisms utilizing such substances or their breakdown products, would antagonize plant pathogens.

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APPENDIX I. CHEMICAL ANALYSIS OF THE THREE ALBERTA SOILS USED IN THESE INVESTIGATIONS.

SOIL	POUNDS PER ACRE AVAILABLE NUTRIENTS: NITRATE NITROGEN (N)	PHOSPHORUS (P)	POTASSIUM (K)	pH	ESTIMATED ORGANIC MATTER LEVEL
G.W.	18	44	120	7.0	very low
Bl.	57	32	180	7.0	medium
Br.	18	32	480	7.8	very low
*	40	60	50	5.8-7.5	high

* A good field soil to plow, depth contains at least

Note: Organic Matter (loss on ashing) Bl. 13.2%, Br. 8.9%, G.W. 6.1%

Results kindly supplied by the Agricultural Soil and Feed Testing Laboratory, University of Alberta, Edmonton.

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